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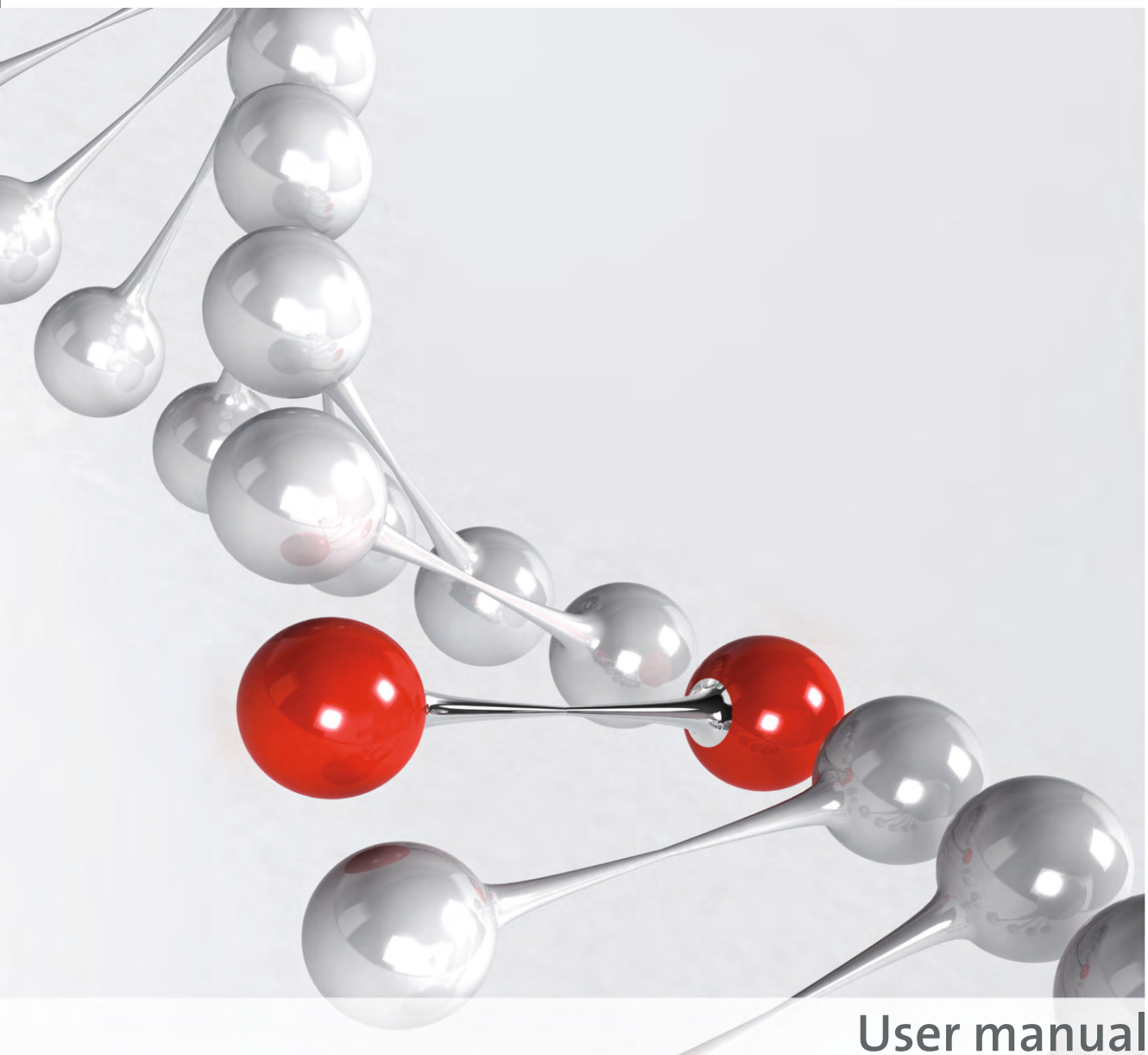

User manual

InviMag® Virus DNA/RNA Mini Kit/ KF96

for use on KingFisher® 96 and KingFisher® Flex, Thermo Fisher

for automated purification of viral DNA and RNA from up to 200 µl serum, plasma, cell culture supernatant and other cell-free body fluids, biopsy samples and swab with magnetic beads

REF 7441050X0

STRATEC Molecular GmbH, D-13125 Berlin

Instruction InviMag® Virus DNA/RNA Mini Kit/ KF96

The **InviMag® Virus DNA/RNA Mini Kit/ KF96** is the ideal tool using the InviMag® technology for simultaneous isolation of DNA and RNA viruses nucleic acids from serum, whole blood, plasma, cerebrospinal fluid, cell culture supernatants and other cell-free body fluids, grind plant material, urine as well as from swabs or tissue biopsies.

The kit is neither validated for the isolation of genomic DNA from stool sample, dried blood stains, nor from bacteria, fungi, parasites.

Trademarks: InviMag®, Invisorb®, RTP®. Registered marks, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

The Invisorb® technology is covered by patents and patent applications: US 6,110363, US 6,043,354, US 6,037,465, EP 0880535, WO 9728171, WO 9534569, EP 0765335, DE 19506887, DE 10041825.2, WO 0034463.

InviMag®, Invisorb® and RTP® are registered trademarks of STRATEC Biomedical AG.

The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

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Kit contents of the InviMag® Virus DNA/RNA Mini Kit/ KF96

Store MAP Solution A at 2-8°C.
Store all other kit components at room temperature.

	1 x 96 extractions	5 x 96 extractions
Catalogue No.	7441050100	7441050200
Extraction Bottle 50 **(incl. Lysis Buffer, Carrier Nucleic Acid, Proteinase K and internal DNA control *)**	2	10
MAP Solution A	2 x 1.2 ml	10.5 ml
Binding Solution (fill with 99.7% isopropanol)	empty bottle (final volume 45 ml)	empty bottle (final volume 220 ml)
Elution Buffer R	15 ml	60 ml
Wash Buffer R1	2 x 30 ml (final volume 2 x 60 ml)	3 x 80 ml (final volume 3 x 160 ml)
Wash Buffer R2	40 ml (final volume 200 ml)	4 x 50 ml (final volume 4 x 250 ml)
Elution Tubes	2 x 50	10 x 50
2.0 ml Deep Well Plate	5	25
KF 96 Tip Comb for DW magnets	1	5
200 µl Elution Plate*	2	5 x 2
Manual	1	1
Initial steps	Add 45 ml **99.7% Isopropanol** (molecular biologic grade) to the empty bottle labeled with “Binding Solution” For **Protocol 1-5** add 10 ml of ddH_2O shortly before use to each Extraction Bottle 50 and mix thoroughly For **Protocol 6** add 17.5 ml of ddH_2O shortly before use to each Extraction Bottle 50 and mix thoroughly Add 30 ml of 96-100% ethanol to each bottle **Wash Buffer R1** and mix thoroughly! Add 160 ml of 96-100% ethanol to the bottle **Wash Buffer R2** and mix thoroughly	Add 220 ml **99.7% Isopropanol** (molecular biologic grade) to the empty bottle labeled with “Binding Solution” For **Protocol 1-5** add 10 ml of ddH_2O shortly before use to each Extraction Bottle 50 and mix thoroughly For **Protocol 6** add 17.5 ml of ddH_2O shortly before use to each Extraction Bottle 50 and mix thoroughly Add 80 ml of 96-100% ethanol to each bottle **Wash Buffer R1** and mix thoroughly Add 200 ml of 96-100% ethanol to each bottle **Wash Buffer R2** and mix thoroughly!

Symbols

Lot number

Catalogue number

Expiry date

Consult operating instructions

Temperature limitation

Do not reuse

Storage

All buffers and kit contents of the **InviMag® Virus DNA/RNA Mini Kit/ KF96,** except **MAP Solution A,** are stable for at least 12 months. **MAP Solution A** is stable for at least 6 months.

All buffers and kit contents of the **InviMag® Virus DNA/RNA Mini Kit/ KF96**, except **MAP Solution A,** should be stored at room temperature (RT).

MAP Solution A should be stored at 2-8°C.

Wash Buffers / Binding buffers charged with either ethanol or isopropanol should be stored at room temperature and must be appropriately sealed. If there are any precipitates visible within the provided solutions, dissolve them by carefully warming up to 30°C.

Room temperature (RT) is defined as range from 15-30°C.

Quality control and product warranty

STRATEC Molecular warrants the correct function of **the InviMag® Virus DNA/RNA Mini Kit/ KF96** for applications as described in this manual. Purchaser must determine the suitability of the product for its particular use. Should any product fail to perform as described in the manual, STRATEC Molecular will check the lot. If STRATEC Molecular investigates a lot connected problem the product will be exchanged free of charge.
STRATEC Molecular reserves the right to change, alter, or modify any product to enhance its performance and design at any time.
In accordance with STRATEC Molecular's ISO 9001-2000 and ISO EN 13485 certified Quality Management System the performance of all components of the **InviMag® Virus DNA/RNA Mini Kit/ KF96** have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.
If you have any questions or problems regarding any aspects of **InviMag® Virus DNA/RNA Mini Kit/ KF96** or other STRATEC Molecular products, please do not hesitate to contact us. A copy of STRATEC Molecular's terms and conditions can be obtained upon request or are presented at the STRATEC Molecular webpage.

For technical support or further information please contact:

from Germany +49-(0)30-9489-2901/ 2910
from abroad +49-(0)30-9489-2907 or contact your local distributor.

Intended use

The **InviMag® Virus DNA/RNA Mini Kit /KF96** is designed for a simultaneous rapid and economical preparation of DNA and RNA from viruses from a wide range of clinical samples, using **RTP® technology**, magnetic beads, and the KingFisher workstation.

The whole process is based on a patented technology, the **InviMag® technology**, for isolation of viral DNA and RNA by binding the nucleic acid onto magnetic particles without chaotropic buffer components.

For reproducible and high yields appropriate sample storage is essential. The purified DNA/RNA is of high quality and can be used for subsequent downstream analysis.

THE PRODUCT IS INDENTED FOR USE BY PROFESSIONALS SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES. It is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of DNA/ RNA followed by signal detection or amplification. Any diagnostic results generated by using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

Product use limitation

The kit is neither validated for the isolation of genomic DNA from stool sample, dried blood stains, nor from bacteria, fungi, parasites or the purification of total RNA.

The included chemicals are only useable once.

If the starting material or the flow trace is changed, no guarantee in operability is issued.

The user is responsible to validate the performance of the STRATEC Molecular product for any particular use. STRATEC Molecular does not provide for validation of performance characteristics of the product with respect to specific applications. STRATEC Molecular products may be used e.g.in clinical diagnostic laboratory systems conditioned upon the complete diagnostic system of the laboratory the laboratory has been validated pursuant to CLIA' 88 regulations in the U.S. or equivalents in other countries.

All products sold by STRATEC Molecular are subject to extensive quality control procedures (according to ISO 9001-2000 and ISO EN 13485) and are warranted to perform as described herein. Any problems, incidents or defects shall be reported to STRATEC Molecular immediately upon their detection.

The chemicals and the plastic parts are for laboratory use only. They must be stored in the laboratory and must not be used for purposes other than intended.

The product with its contents is unfit for consumption.

Safety information

When and while working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles!

Avoid direct skin contact! Adhere to the legal requirements for working with biological material!

For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at **www.stratec.com** for each STRATEC Molecular product and its components. If buffer bottles are damaged or leaking, WEAR GLOVES AND PROTECTIVE GOGGLES when discarding the bottles in order to avoid any injuries.

STRATEC Molecular has not tested the liquid waste generated by the **InviMag® Virus DNA/RNA Kit/ KF96** procedures for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely, but cannot be excluded completely. Therefore, liquid waste must be considered infectious and be handled and discarded accordingly to local safety regulations.

European Community risk and safety phrases for the components of the InviMag® Virus DNA/RNA Mini Kit/ KF96 to which they apply, are listed below as follows.

Wash Buffer R1 contains Guanidinthiocyanate which is an irritant. Wash Buffer and Elution Buffer R contain DEPC-treated water.

Proteinase K (included in the **Extraction Tube**)

danger
H315-319-334-335 P280-305-351-338-310-405

Wash Buffer R1

danger
H302-312-332-412 EUH032 P273

H319:	Causes serious eye irritation.
H302:	Harmful if swallowed.
H312:	Harmful in contact with skin.
H332:	Harmful if inhaled.
H412:	Harmful to aquatic life with long lasting effects.
EUH032:	Contact with acids liberates very toxic gas.
H315:	Causes skin irritation.
H334:	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335:	May cause respiratory irritation.
P305+P351+P338:	If in eyes: Rinse cautiously with water for several minutes. If present, remove contact and continue rinsing.
P273:	Avoid release to the environment.
P280:	Wear protective gloves/protective clothing/eye protection/face protection.
P310:	Immediately call a POISON CENTER or doctor/physician.
P405:	Store locked up.

Emergency medical information can be obtained 24 hours a day from infotrac:

outside of USA: 1 – 352 – 323 – 3500
inside of USA : 1 – 800 – 535 – 5053

Product characteristic of the InviMag® Virus DNA/RNA Mini Kit/ KF96

Starting material	Yield	Time for preparation
up to 200 µl fresh or frozen plasma, serum and cell free body fluids up to 200 µl cell culture supernatant up to 200 µl of rinse liquid from swabs up to 50 µl whole blood up to 10 mg biopsy samples up to 50 µl grind plant material	Sensitive recovery realizing amplification with sensitive detection assays from starting material including minimal 100 copies / ml	about 35 min (without lysis)

The **InviMag® Virus DNA/RNA Mini Kit/ KF96** is designed for simultaneous, rapid and economical preparation of viral DNA and RNA from a wide range of samples, using a combination of RTP® technology, magnetic beads and the KF96 / KFflex96 workstation.

The isolation process is based on a patented technology, the InviMag® technology, for isolation of viral RNA and DNA by binding the nucleic acid onto magnetic particles without chaotropic buffer components.

The sample is lysed in an optimized lysis buffer. The lysates are transferred to the subsequent automated purification procedure based on magnetic beads. The viral DNA and RNA bind to magnetic particles, followed by washing steps and the final elution. The procedure requires only minimal interaction by the user, allowing safe handling of potentially infectious samples. The procedures are designed to avoid sample-to-sample cross-contamination.

The purified high quality viral DNA and RNA is ready-to-use for subsequent downstream applications (see below) or can be stored at -20°C for subsequent use.

- RT-PCR*
- PCR*
- Real-time PCR for quantitative and qualitative virus detection

No toxic or hazardous chemicals like chaotropic components are used.

For the isolation of viral nucleic acids from single sample volumes from up to 200 µl of starting material STRATEC Molecular offers the **RTP® DNA/RNA Virus Mini Kit** and the **Invisorb® Spin Virus RNA Mini Kit**. For the isolation of viral nucleic acids using magnetic particle in low-throughput format, STRATEC Molecular offers the **InviMag® Virus Kits/ KFmL** for use on a KingFisher mL workstation. For vacuum or centrifuge based isolation of viral NA in a convenient 96 well format on different robotic stations STRATEC Molecular offers the **Invisorb® DNA Virus HTS 96 Kit** and the **Invisorb® RNA Virus HTS 96 Kit** (see ordering information, page 24)
For further information please contact: +49 (0) 30 9489 2901, 2910 in Germany and from foreign countries: +49 (0) 30 9489 2907 or call your local distributor.

Principle and procedure

The **InviMag® Virus DNA/RNA Mini Kit /KF96** procedure comprises following steps:
- preparation of the Lysis Buffer in a prefilled Extraction Bottle
- lysis of the virus particles in the Lysis Buffer in a 96 well plate
- binding of the viral NA to the magnetic beads
- washing of the viral NA bound on magnetic beads and elimination of ethanol
- elution of the viral nucleic acids

After lysis, the viral nucleic acids bind to the added magnetic beads whereas contaminations and enzyme inhibitors are efficiently removed during the following three washing steps. Highly purified DNA-RNA is finally eluted in Elution Buffer R.
This manual contains 6 protocols.

* The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

Lysis
Samples are lysed at denaturing conditions using elevated temperatures.

Binding of the viral nucleic acids
After adding **Binding Solution** and **MAP Solution A** to the lysate in a **2 ml Deep Well Plate**, the nucleic acids are bound to the surface of the magnetic beads.

Removing residual contaminants
Contaminants are efficiently removed using **Wash Buffer R1** and **R2**, while the nucleic acids remain bound to the magnetic beads

Elution
The nucleic acids are eluted from the beads using **Elution Buffer R**. The eluted nucleic acids are ready-to-use in different subsequent tests.

Yield and quality of viral DNA and / or viral RNA

Yield and quality of isolated viral DNA and RNA is suitable for any virus detection system. The tests should be performed accordingly to the manufacturer's specifications. The amount of purified viral nucleic acids derived by the **InviMag® Virus DNA/RNA Mini Kit/ KF96** procedure from plasma etc. depends on the sample type, sample source, transport, storage and age.

Different amplification systems vary in efficiency depending on the total amount of nucleic acids present in the reaction. Eluates derived by this kit will contain Carrier-RNA, which will greatly exceed the amount of the isolated NA.

Yields of viral nucleic acids isolated from biological samples are usually low concentrated and therefore almost impossible to determine photometrically.*

* Keep in mind that the Carrier-RNA (5 µg per 200 µl sample) will account for most of the present RNA.

The kit is suitable for downstream analysis using common nucleic acid analysis techniques like qPCR, RT qPCR, LAMP, LCR. Diagnostic assays should be performed accordingly to the manufacturer's instructions.

Quantitative RT-PCR or real time PCR is recommended for determination of viral nucleic acid yield.

* In Gel Electrophoresis and in Capillary Electrophoresis, RNA extracted with the provided kit looks like degraded cause the kit contains Carrier-RNA, this is poly A RNA in fragments of 100 up to 1000 bases. The kit is not dedicated for applications using this kind of analysis.

Sampling and storage of starting material

Serum, plasma, urine, synovial fluid samples or other cell-free body fluids and swabs can be stored on ice for up 1-2 hours. For short time (up to 24 h) samples may be stored at -20°C. For long-term storage, we recommend freezing the samples at -80°C. Multiple thawing and freezing cycles before isolating the viral nucleic acids should be avoided.

STRATEC Molecular will be released of its responsibilities if other sample materials than described in the Intended Use are processed or if the sample preparation protocols are changed or modified.

Important notes

Important points before starting a protocol

Immediately upon receipt of the product, inspect the product and its components as well as the package for any apparent damages, correct quantities and quality. If there are any unconformities you have to notify STRATEC Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to "Safety Information" (see page 6). Do not use damaged kit components because their use may lead to poor kit performance.

- Always change pipet tips between liquid transfers. To avoid cross-contamination, we recommend the use of aerosol-barrier pipet tips.
- All centrifugation steps are carried out at room temperature.
- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.
- Discard contaminated gloves immediately.
- Do not combine components of different kit, unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar air-flow until the samples are lysed.
- The kit should only be used by trained personnel.

Internal control (IC) / Extraction control

Internal controls (IC) from the PCR assay provider can be used as extraction controls. In this case they have to be added to the lysate of the clinical sample following the instructions after finalization of the lysis step. If it only should be used as inhibition control it has to be added to the final eluted RNA/DNA.

Attention: Don´t add the internal controls directly to the sample!

Preparing reagents and buffers

1x 96 DNA-extractions:
Add 45 ml 99.7% Isopropanol (molecular biologic grade) to the empty bottle labeled with "Binding Solution"
Add 10 ml ddH_2O (17.5 ml for protocol 6) shortly before use to each Extraction Bottle 50 and mix thoroughly
Add 30 ml of 96-100% ethanol to each bottle Wash Buffer R1,mix thoroughly and always keep the bottle firmly closed
Add 160 ml of 96-100% ethanol to the bottle Wash Buffer R2, mix thoroughly and always keep the bottle firmly closed
5x 96 DNA-extractions:
Add 220 ml 99.7% Isopropanol (molecular biologic grade) to the empty bottle labeled with "Binding Solution"
Add 10 ml ddH_2O (17.5 ml for protocol 6) shortly before use to each Extraction Bottle 50 and mix thoroughly
Add 80 ml of 96-100% ethanol to each bottle Wash Buffer R1, mix thoroughly and always keep the bottle firmly closed
Add 200 ml of 96-100% ethanol to each bottle Wash Buffer R2, mix thoroughly and always keep the bottle firmly closed

Reagents and equipment to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS). (See our webpage: **www.stratec.com**)

- Measuring cylinder (250 ml)
- Pipette and pipette tips
- Disposable gloves
- Reaction tubes (1.5 ml or 2.0 ml)
- ddH_2O
- Vortexer
- 96–100% ethanol
- 99.7% isopropanol *

*The **InviMag® Virus DNA/RNA Mini Kit /KF96** is validated with 2-Propanol; Rotipuran >99.7%, p.a., ACS, ISO (Order no. 6752) from **Carl Roth.**

*** Possible suppliers for Isopropanol:**

Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order No. 6752

Applichem
2-Propanol, molecular biology grade
Order No. A3928

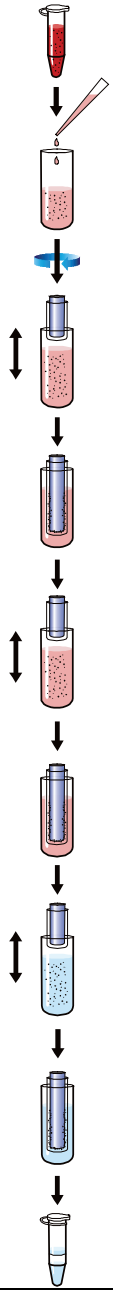
Sigma Aldrich
2-Propanol
Order No. 59304-1L-F

Scheme of the InviMag® Virus DNA/RNA Mini Kit/ KF96

Please read protocols prior the start of the preparation carefully

Prepare the Lysis Solution Mixture following the extraction protocols on page 11-15.
Add 200 µl of this **Lysis Solution Mixture** to each cavity of the Binding Plate* and 200 µl sample.
During lysis, prefill all plates with required buffers and appropriate volumes. Add **Internal Control (IC)** to each sample after the lysis step.

Tip Plate:	Insert the KF96 Tip Comb for DW magnets on a Tip Plate*
Binding Plate*:	Add 400 µl **Binding Solution** and 20 µl **MAP Solution A** after lysis
Washing Plate_1*:	Add 800 µl **Wash Buffer R1** to a 2.0 ml Deep Well Plate
Washing Plate_2*:	Add 800 µl **Wash Buffer R2** to a 2.0 ml Deep Well Plate
Washing Plate_3*:	Add 800 µl **Wash Buffer R2** to a 2.0 ml Deep Well Plate
Elution Plate:	Add 100 µl **Elution Buffer R** to the KF Elution Plate (same size as Tip Plate)



Please read the protocols carefully prior to the start of the preparation procedure!

Sample transfer into the Lysis/ Binding Plate prefilled with extraction solution

First, two heating steps are performed.

After lysis, a pause occurs and 400 µ **Binding Solution** and 20 µl **MAP Solution A** are added to the Lysis/Binding Plate

Nucleic acids bind to magnetic particles

Washing of the particle fixed nucleic acids

Magnetic separation

Elution of viral DNA / RNA

Magnetic Separation

Pure nucleic acids

* Elution Plates and Tip Plates are identically. Use one provided Elution Plate as a Tip Plate.

Protocol 1: Simultaneous isolation of total nucleic acids (viral DNA and RNA) from cell-free body fluids (serum, plasma, CSF, synovial, urine)

Please read the instructions carefully and conduct the prepared procedure.

Preparation of Lysis Solution Mixture
Add 10 ml ddH_2O to each Extraction Bottle 50 before use and mix thoroughly.

Sample Lysis
Transfer 200 µl **Lysis Solution Mixture** from the **Extraction Bottle 50** to a cavity of a **Binding Plate** (2 ml Deep Well Plate) and add 200 µl sample. Mix by pipetting up and down or close the plate with a sealing foil and mix by vortexing for 10 s

Note ***If the sample volume is less than 200 µl use distilled water or 1x PBS. The final sample volume must be 200 µl***

Place the sealed **Binding Plate** (2 ml Deep Well Plate) onto a Thermomixer and incubate while continuously shaking for 15 min at 65°C. During lysis step, prefill all plates with the required buffers and appropriate volumes (see “Starting a run on a KF96/ KFflex96 instrument” page 16).

Note ***Mixing during lysis is essential!***

After the first lysis step, transfer the **Binding Plate** onto another Thermomixer and incubate for 10 min at 95°C while continuously shaking.
(This step leads to a higher sensitivity for some viruses or strains of viruses e.g. HIV or HCV.)

Incubate the plate for 10 min at RT to cool down the plate.

Add the **Internal Control** (IC) to each sample.

Add 400 µl of **Binding Solution** and 20 µl **MAP Solution A** to each sample containing well and start the assay “InviMag Virus DNA-RNA KFflex96” or “InviMag Virus DNA-RNA KF96” (see page 16 for further details).

Protocol 2: Simultaneous isolation of total nucleic acids (viral DNA and RNA) from whole blood

Please read the instructions carefully and conduct the prepared procedure.

Preparation of Lysis Solution Mixture
Add 10 ml ddH_2O to each Extraction Bottle 50 before use and mix thoroughly.

Sample Lysis
Transfer 200 µl **Lysis Solution Mixture** from the **Extraction Bottle 50** to a cavity of a **Binding Plate** (2 ml Deep Well Plate) and add 50 µl sample and 150 µl ddH_2O to the same cavity. Mix by pipetting up and down or close the plate with a sealing foil and mix by vortexing for 10 s**.**

Place the sealed **Binding Plate** (2 ml Deep Well Plate) onto a Thermomixer and incubate while continuously shaking for 15 min at 65°C. During the lysis step, prefill all plates with the appropriate buffers and volumes (see "Starting a run on a KF96/ KFflex96 instrument" page 14).

Note **Mixing during lysis is essential!**

After the first lysis step, transfer the **Binding Plate** (2 ml Deep Well Plate) onto another Thermomixer and incubate for 10 min at 95°C while continuously shaking.
(This step leads to higher sensitivity for some viruses or strains of viruses e.g. HIV or HCV.)

Incubate the plate for 10 min at RT to cool down the plate.
Add the **Internal Control** (IC) to each sample.

Add 400 µl of **Binding Solution** and 20 µl **MAP Solution A** to each sample containing cavity and start the assay "InviMag Virus DNA-RNA KFflex96" or "InviMag Virus DNA-RNA KF96" (see page 16 for further details).

Protocol 3: Simultaneous isolation of total nucleic acids (viral DNA and RNA) from cell culture supernatant

Please read the instructions carefully and conduct the prepared procedure.

Preparation of Lysis Solution Mixture

Add 10 ml ddH_2O to each Extraction Bottle 50 before use and mix thoroughly.

Sample Lysis
Transfer 200 µl **Lysis Solution Mixture** from the **Extraction Bottle 50** to a cavity of a **Binding Plate** (2 ml Deep Well Plate) and add 200 µl cell free cell culture supernatant (cell culture media). Mix by pipetting up and down or by vortexing. Close the plate with a sealing foil before mixing.

Note ***If the sample volume is less than 200 µl use distilled water or 1x PBS. The final sample volume must be 200 µl***

Place the sealed **Binding Plate** (2 ml Deep Well Plate) onto a Thermomixer and incubate while continuously shaking for 15 min at 65°C. During the lysis step, prefill all plates with the appropriate buffers and volumes (see "Starting a run on a KF96/ KFflex96 instrument" page 14).

Note **Mixing during lysis is essential!**

After the first lysis step, transfer **Binding Plate** (2 ml Deep Well Plate) onto another Thermomixer and incubate for 10 min at 95°C while continuously shaking.
(This step leads to higher sensitivity for some viruses or strains of viruses e.g. HIV or HCV.)

Incubate the plate for 10 min at RT to cool down.
Add the **Internal Control** (IC) to each sample.

Add 400 µl **Binding Solution** and 20 µl **MAP Solution A** to each sample containing cavity and start the assay "InviMag Virus DNA/RNA KFflex96" or "InviMag Virus DNA-RNA KF96" (see page 16 for further details).

Protocol 4: Simultaneous isolation of total nucleic acids (viral DNA and RNA) from swabs or rinsed liquid

Please read the instructions carefully and conduct the prepared procedure

Preparation of Lysis Solution Mixture

Add 10 ml ddH_2O to each Extraction Bottle 50 before use and mix thoroughly.

Sample Lysis

Place a swab into a cavity of a **Binding Plate** (2 ml Deep Well Plate) and add 200 µl ddH_2O and 200 µl Lysis Solution Mixture from the **Extraction Bottle**. Firmly close the plate / reaction tube and mix by vortexing for 10 s. In order to be able to close the tube / DWP cut off the shaft from the swab.

Note: *If the sample volume is less than 200 µl use distilled water or 1x PBS. The final sample volume must be 200 µl*

Place the sealed **Binding Plate** (2 ml Deep Well Plate) onto a Thermomixer and incubate for 15 min at 65°C while continuously shaking. During the lysis step, prefill all plates with the appropriate buffers and volumes (see “Starting a run on a KF96/ KFflex96 instrument” page 14).

Note: *Mixing during lysis is essential!*

After the first lysis step, transfer **Binding Plate** (2 ml Deep Well Plate) to another Thermomixer and incubate while continuously shaking for 10 min at 95°C.
(These step leads to higher sensitivity for some viruses or strains of viruses e.g. HIV or HCV.)

Incubate the plate for 10 min at RT to cool down.

Add the **Internal Control** (IC) to each sample.

After the second lysis step, carefully take out the swab and squeeze it out inside the cavity of the DWP / reaction tube. Discard the swab and completely.
Add 400 µl **Binding Solution** and 20 µl **MAP Solution A**. Start the assay “InviMag Virus DNA-RNA KFflex96” or “InviMag Virus DNA-RNA KF96” (see page 16 for further details).

Protocol 5: Simultaneous isolation of total nucleic acids (viral DNA and RNA) from tissue biopsy

Please read the instructions carefully and conduct the prepared procedure

Preparation of Lysis Solution Mixture
Add 10 ml ddH_2O to each Extraction Bottle 50 before use and mix thoroughly.

Sample Lysis
Transfer 1-10 mg of the tissue biopsy sample into a cavity of a 2 ml Deep Well Plate and add 180 µl ddH_2O and 200 µl Lysis Solution Mixture of the **Extraction Bottle.** Close/Seal the reaction tube / DWP and mix by vortexing for 10 s.

Place the sealed 2 ml Deep Well Plate onto a Thermomixer and incubate for 15 min at 65°C while continuously shaking. The lysis time can be increased for up to 30 min. However, a prolonged lysis time may reduce the final yield and quality of some viral RNA species.

Note: *Mixing during lysis is essential!*

During the first lysis step, prefill all plates with the appropriate buffers and volumes as indicated on page 16.

After the first lysis step, transfer the 2 ml Deep Well Plate to another Thermomixer and incubate while continuously shaking for 10 min at 95°C.
(These step leads to higher sensitivity for some viruses or strains of viruses e.g. HIV or HCV.)

After lysis, centrifuge the lysed sample(s) at max. speed for 1 min to spin down unlysed material. Transfer the supernatant into the **Binding Plate** (2 ml Deep Well Plate) prefilled with 400 µl **Binding Solution** and 20 µl **MAP Solution A** and start the assay "InviMag Virus DNA/RNA KFflex 96" or "InviMag Virus DNA-RNA KF96" (see page 16 for further details).

Protocol 6: Simultaneous isolation of total nucleic acids (viral DNA and RNA) from grind plant material

Please read the instructions carefully and conduct the prepared procedure.

Preparation of Lysis Solution Mixture
Add 17.5 ml ddH_2O to each Extraction Bottle 50 before use and mix thoroughly.

Sample Lysis
Transfer 350 µl Lysis Solution Mixture from the **Extraction Bottle 50** to a free cavity of a 2 ml Deep Well Plate and add 50 µl grinded plant material dissolved in buffer used for shredding. Mix by pipetting up and down or by vortexing. If the plate must be vortexed, ensure that the plate is properly sealed with a sealing foil to prevent cross-contaminations.

Place the sealed 2 ml Deep Well Plate onto a Thermomixer and incubate for 15 min at 65°C while continuously shaking. During the first lysis step, prefill all plates with the appropriate buffers and volumes (see "Starting a run on the KF96 / KFflex96 instrument" on page 16).

Note **Mixing during lysis is essential!**

After the first lysis step, transfer the liquid from the 2 ml Deep Well Plate onto another Thermomixer and incubate for 10 min at 95°C while continuously shaking.
(This step leads to higher sensitivity for some viruses or strains of viruses e.g. HIV or HCV.)

After the second lysis step, carefully transfer the lysed sample(s) into the **Binding Plate (**2 ml Deep Well Plate) prefilled with 400 µl **Binding Solution** and 20 µl **MAP Solution A** and start the assay "**InviMag Virus DNA-RNA KFflex96**" or "**InviMag Virus DNA-RNA KF96**" (see page 16 for further details) depending on the used instrument.

Starting a Run on a KF96 / KFflex96 instrument

I. Preliminary Steps to process the sample onto the KF96 / KFflex96 System

Important: *For working with the King Fisher instruments, please carefully read the manufacturer's manual!*

1. Turn on the KF96 / KFflex96 instrument

a. **Tip Plate**: Place the KF96 Tip Comb for DW magnets on an Elution Plate (Tip Plate).

Note*: Use one provided Elution Plate as Tip Plate. These are identical.*

2. Prefill the Deep Well Plates with the required buffers and appropriate volumes

Note*: In case of long waiting times, please avoid evaporation of the prefilled buffer components by sealing the Deep Well Plates with a sealing foil or with parafilm!*

Important: *Mix the bottle with the **MAP Solution A** by vigorously vortexing!*

Binding Plate:	Add 400 µl **Binding Solution** and 20 µl **MAP Solution A**
Washing plate_1:	Add 800 µl **Wash Buffer R1** into the cavities of a 2 ml Deep Well Plate
Washing plate_2:	Add 800 µl **Wash Buffer R2** into the cavities of a 2 ml Deep Well Plate
Washing plate_3:	Add 800 µl **Wash Buffer R2** into the cavities of a 2 ml Deep Well Plate
Elution Plate:	Add 100 µl **Elution Buffer R** into the cavities of the KF Elution Plate

3. After addition of all solutions choose the running file **"InviMag Virus DNA-RNA KFflex96"** or **"InviMag Virus DNA-RNA KF96"** depending on the instrument you are using on the display of the instrument and press the "START" button.
4. Insert the prefilled plates into the right position of the instrument surface by following the specification printed on display and confirm every step by pressing the "START" button.
5. After all prefilled plates have been added to the system press the "START" button again to initialize the run.

II. The following steps run automatically on the KingFisher System

1. Binding of the DNA
Binding of the isolated nucleic acids for 5 min. Transportation of the MAP bound nucleic acids into Washing Plate 1.

2. First Washing Step
Automatically sample washing for 1 min. MAP separation. Transportation of the MAPs bound nucleic acids into the Washing Plate 2.

3. Second Washing Step
Automatically sample washing for 1 min. MAP separation. Transportation of the MAP bound nucleic acids into the Washing Plate 3.

4. Third Washing and Drying Step
Automatically sample washing for 1 min. MAP separation. Drying of the MAP bound nucleic acids outside the plate for 8 minutes. Transportation of MAP into the Elution Plate.

5. Elution of the nucleic acids
Incubation of the MAP bound nucleic acids into the Elution Plate for 10 minutes at 60°C by mixing. MAP separation. The MAP without the bound nucleic acids are then automatically be removed into Washing Plate_3 (disposal).

Important Notes:

After finishing the extraction protocol, the Elution plate contains the extracted viral RNA / DNA. Store the RNA / DNA at adequate conditions. We recommend storing the samples at -20°C or -80°C.
If the viral RNA / DNA contains carryover from magnetic particles, transfer the eluate into a 1.5 ml reaction tube and centrifuge at maximum speed (13.000 rpm) for 1 min. Transfer the viral RNA/DNA containing supernatant into a new tube.

For self-programming of the KF96 and KFflex96 instrument

Reagent info

Tip Plate		KingFisher 96 KF plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
-	-	-	-

Binding Plate		Microtiter DW 96 plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Lysed sample	400	-	Sample
Binding Solution	400	-	Reagent
MAP Solution A	20	-	Reagent

Washing Plate 1		Microtiter DW 96 plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer R1	800	-	Reagent

Washing Plate 2		Microtiter DW 96 plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer R2	800	-	Reagent

Washing Plate 3		Microtiter DW 96 plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer R2	800	-	Sample

Elution Plate		KingFisher 96 KF plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Elution Buffer R	100	-	Reagent

Dispensed reagents

The protocol does not contain dispensed reagents

Steps data

Tip1		96 DW tip comb	
	Pick-Up	Tip Plate	
	Binding	Binding Plate	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Medium
	Mixing / heating:	Mixing time, speed	00:05:00, Medium
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5
	Washing_1	Washing Plate 1	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	3
		Collect time [s]	5
	Washing_2	Washing Plate 2	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	3
		Collect time [s]	5
	Washing_3	Washing Plate 3	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	3
		Collect time [s]	5
	Drying	Washing Plate 3	
		Dry time	00:08:00
		Tip position	Outside well / tube

	Elution	Elution Plate	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Mixing time, speed	00:10:00, Slow
		Heating temperature [°C]	60
		Preheat	Yes
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5
	Bead Removal	Washing Plate 3	
		Release time, speed	00:00:30, Fast
	Leave	Tip Plate	

Troubleshooting

Problem	Probable cause	Comments and suggestions
low amount of extracted DNA/RNA	insufficient lysis	increase lysis time, but prevent too long lysis because this also decrease yield reduce amount of starting material
	incomplete elution	use higher volume of **Elution Buffer R**. Ensure that the **Elution Buffer R** is transferred to the correct plate
	low amount of **MAP Solution A**	mix **MAP Solution A** thoroughly before pipetting into the DWP
low concentration of extracted DNA/RNA	too much Elution Buffer	elute the DNA with a lower volume of **Elution Buffer R**
	incorrect storage of starting material	ensure that the storage of starting material was correctly avoid multiple thawing and freezing cycles of the material
degraded or sheared DNA/RNA	incorrect storage of starting material	ensure that the storage of starting material was correctly avoid thawing of the material
	old material	ensure that the starting material is fresh or stored under appropriate conditions (storage at –20°C)! avoid multiple thawing and freezing cycles of the material old material often contains degraded DNA / RNA
DNA/RNA does not perform well in downstream applications (e.g. real-time PCR or PCR)	ethanol carryover during elution	increase drying time for removing of ethanol
	salt carryover during elution	check the **Wash Buffers** for salt precipitates. If there are any precipitates visible, solve them by carefully warming up to 30°C ensure that the **Wash Buffers** are at room temperature
low A_{260}:A_{280} ratio from UV measurement, eluted DNA/RNA is brown colored	small part of the magnetic particles are left in the elution	centrifuge at full speed for 1 min and transfer supernatant to a new tube

Appendix

KingFisher BindIt Software 3.2 or higher versions

BindIt software 3.2 or higher versions were and may be used to create assay files for the KFmL, KF96/KFflex96 or KF-Duo instruments. The provided assay file(s) can either be transferred onto the corresponding workstation(s) or be started directly from within the BindIt software after assay import. Please keep in mind, that assay(s) run from within the BindIt software are not stored in the workstation memory.

Important: *Be advised that BindIt SW 3.2 or higher versions use a new unique file extension. Therefore, it is not possible to import assay files created with BindIt 3.2 or higher versions into older BindIt software versions! Please ask your local Thermo Scientific distributor for a software update.*

Note*:* *When creating assay files for usage with KingFisher instruments in combination with Microtiter Deep Well plates (e.g. Thermo Electron), it is essential to use the KingFisher software 3.2 or higher versions for assay development because this software version includes the correct adjustments for the microtiter plate. It is highly recommended to use Thermo Microtiter Deep Well plates with KF96 / KFflex96 / KF-Duo workstations to ensure the best purification result.*

Minimum system requirements for BindIt Software 3.2 or higher versions

PC requirements	
Supported operating systems	MS Windows XP Pro with SP3, Windows Vista SP2, Windows 7
Disk space	500 MB free disk space
Processor	Intel Pentium ≥ 1 GHz
Memory	1 GB RAM
Serial ports available	1 (for KFmL connection)
USB ports available	1 (for KF96 / KFflex96 / KFDuo connection)
Pointing device	Mouse or equivalent is required
CD-ROM drive	1
Monitor / color settings	XVGA monitor with at least 1024x768 resolution and at least a 16-bit color environment

If the actual Windows Service Packs are not installed on the corresponding lab computer, they can be downloaded from the Microsoft web pages: http://www.microsoft.com/

General notes on handling DNA

Nature of DNA
The length and delicate physical nature of DNA requires careful handling to avoid damage due to shearing and enzymatic degradation. Other conditions that affect the integrity and stability of DNA include acidic and alkaline environments, high temperature, and UV irradiation. Careful isolation and handling of high molecular weight DNA is necessary to ensure compatibility with various downstream applications. Damaged DNA could perform poorly in applications such as genomic Southern blotting, long template PCR.

Storage of DNA
A working stock of DNA can be stored at 2-8˚C for several weeks. For long-term storage DNA should be stored at -20˚C, but storing at -20°C can cause shearing, particularly if the DNA is exposed to repeated freeze / thawing cycles.

Note that the solution in which the nucleic acid is eluted in will affect it's stability during storage. Pure water lacks buffering capacity and an acidic pH may lead to acid hydrolysis. Tris or Tris-EDTA buffer contains sufficient buffering capacity to prevent acid hydrolysis.

Drying, dissolving and pipetting DNA
Avoid overdrying genomic DNA after ethanol precipitation. It is better to let it air dry than to use a vacuum, although vacuum drying can be used with caution.
Avoid vigorous pipetting. Pipetting genomic DNA through small tip openings causes shearing or nicking. One way to decrease shearing of genomic DNA is to use special tips that have wide openings designed for pipetting genomic DNA.

DNA Yield
The amount of purified viral DNA depends on sample source, transport conditions, storage, and age of the sample.

General notes on handling viral RNA

RNA is far less stable than DNA. It is very sensitive to degradation by endogenous RNases in the biological material and exogenous RNases which are permanently present everywhere in the lab. To achieve satisfactory, qualitative and quantitative results in RNA preparations, contaminations with exogenous RNases have to be reduced as much as possible Avoid handling bacterial cultures, cell cultures or other biological sources of RNases in the same lab where the RNA purification is carried out.

All glassware should be treated before use to ensure a RNase-free environment. Glassware should be cleaned with detergent, thoroughly rinsed and oven baked at 240°C for four or more hours before use. Autoclaving alone will not completely inactivate many RNases. Oven baking will both inactivate RNases and ensure that no other nucleic acids (such as Plasmid DNA) are present on the surface of the glassware. You can also clean glassware with 0.1% DEPC (diethyl pyrocarbonate). The glassware must stand 12 hours at 37°C and then be autoclaved or heated to 100°C for 15 min to remove residual DEPC.

- **Electrophoresis tanks** should be cleaned with detergent solution (e.g. 0.5% SDS), thoroughly rinsed with RNase-free water, and then rinsed with ethanol and allowed to dry.
- **Non-disposable plastic ware** should be treated before use to ensure that it is RNase-free. Plastic ware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA followed by RNase-free water. A use of chloroform resistant plastic ware rinsed with chloroform to inactivate RNases is recommended.
- All **buffers** must be prepared with DEPC-treated RNase-free ddH_2O.
- When **working with chemicals**, always wear a suitable lab coat, disposable gloves, and protective goggles.
- Change gloves frequently and keep tubes closed.
- All centrifugation steps are carried out at room temperature.
- To avoid **cross-contamination** cavity seams shouldn't be moisten with fluid.
- Reduce the preparation time as much as possible.
- **Use only sterile, disposable polypropylene tubes** throughout the procedure. (These tubes are generally RNase-free.)
- Always keep isolated RNA on ice.
- Do not merge kit components from other kits, unless the lot numbers are identical.
- To minimize the risk of infections from potentially pathogen material, we recommend working under laminar airflow until the samples are lysed.

This kit should only be used by trained personnel.

Storage of viral RNA

Purified viral RNA can be stored at -80°C and is stable for several years at this condition.

Ordering Information

Product	Package Size	Catalogue No.
InviMag® Virus DNA/RNA Mini Kit/ KF96	15 preparations	7441050100
InviMag® Virus DNA/RNA Mini Kit/ KF96	75 preparations	7441050200

Related products

InviMag® Virus DNA/RNA Mini Kit/ KFmL	15 preparations	2441150100
InviMag® Virus DNA/RNA Mini Kit/ KFmL	75 preparations	2441150200
InviMag® Virus DNA/RNA Mini Kit/ KFmL	300 preparations	2441150400
InviMag® Virus DNA/RNA Mini Kit	50 preparations	1440100200
InviMag® Virus DNA/RNA Mini Kit	250 preparations	1440100300
InviMag® Virus DNA Kit/ KF96	1 x 96 preparations	7440300100
InviMag® Virus DNA Kit/ KF96	5 x 96 preparations	7440300200
InviMag® Virus RNA Kit/ KF96	1 x 96 preparations	7443300100
InviMag® Virus RNA Kit/ KF96	5 x 96 preparations	7443300200
RTP® DNA/RNA Virus Mini Kit	50 preparations	1040100200
RTP® DNA/RNA Virus Mini Kit	250 preparations	1040100300
Invisorb® Virus DNA HTS 96 Kit /X	4 x 96 preparations	7142310300
Invisorb® Virus DNA HTS 96 Kit /X	24 x 96 preparations	7142310400
Invisorb® Virus RNA HTS 96 Kit /X	4 x 96 preparations	7143310300
Invisorb® Virus RNA HTS 96 Kit /X	24 x 96 preparations	7143310400

KingFisher 96 and consumables

KingFisher 96, Magnetic Particle Processor,100-240V,50/60Hz (including one magnetic head)	5400500
KingFisher 96 Head for Deep Well plate	24073430
KingFisher 96 tip comb for PCR magnets, 8 x 10 pcs / box	97002514
KingFisher 96 tip comb for KF magnets, 10 x 10 pcs / box	97002524
KingFisher 96 tip comb for DW magnets 10 x 10 pcs / box	97002534
KingFisher 96 KF plate (200ul) 48 plates / box	97002540
Microtiter deep well 96 plate, 50 plates/box	95040450

Possible suppliers for Isopropanol:

Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order No. 6752

Applichem
2-Propanol, molecular biology grade
Order No. A3928

Sigma-Aldrich
2-Propanol
Order No. 59304-1L-F

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